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ANTIBACTERIAL ACTIVITY OF LICHEN *PARMOTREMA* SPP.

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ABSTRACT

An attempt has been made to study the antibacterial activity of different lichen *Parmotrema* spp. and subjected for solvent extraction against seven clinically potential antibiotic resistant bacteria. Different types of *Parmotrema* spp. such as *P. austrosinense, P. hababianum, P. nilgherrense. P. reticulatam* and *P. tinctorum* were collected from various living and non-living substrates Kodaikanal hills of Tamil Nadu, India for the present study. Antibiotic like compounds were successfully extracted from the thallus of *Parmotrema* spp. using petroleum ether, ethyl acetate and chloroform subsequently tested for their antibacterial activity by well diffusion method. The results showed that a remarkable antibacterial activity of all *Parmotrema* spp. against the tested organisms was recorded. But antibacterial activity of *Parmotrema* spp. was found to be inferior to the standard antibiotics such as streptomycin and ciprofloxacin. The results further revealed that there was no significant difference among *Parmotrema* spp. tested in terms of antibacterial activity. It was due to several biotic and abiotic factors including origin of plant materials, growth habitats, vegetation habits, age of the plant materials and extract preparation. Ethyl acetate extract of tea leaves was found to be the most effective against human pathogenic organisms in terms of growth inhibition.

Keywords: Lichen, *Parmotrema* spp., Antibacterial activity, Antibiotics, Pathogenic bacteria, Clinical pathogenic organisms.

INTRODUCTION

The chemotherapeutic agents are playing important role in green pharmacy due to immediate mode of action and metabolism against clinically potential antibiotic resistant pathogenic organisms. In addition, traditional medicine systems is found to be the best and also serve the health need of about 80% of the world's population. It is in accordance with rapid metabolic action and less adverse effect (WHO, 2013). Most of the traditional system of medicine is being prepared from herbal plants. These traditional novel drug molecules are very active against most of the clinically potential antibiotic resistant pathogenic organisms. According to Ponmurugan *et al* (2016) the increasing concentration of drugs towards green pharmacy may be due to emergence of antibiotic resistance organisms, side effects, rapid mode of action and economic concern too. Importance and the alarming situation on the steady increase of antibiotic resistance microorganisms throughout the world against find out new drug molecules, which resulted increased illness followed by deaths (Levy, 2002) and highlighting the search for a novel antimicrobial agents against challenging human pathogens (Stepanovic *et al*., 2003). Moreover, the challenge for today's pharmaceutical industries is purely based on the discovery and development of new pharmacological active molecules from medicinally important plants (Behera *et al*., 2005). Antimicrobial activity of medicinally important plants against numerous human pathogenic microorganisms are already reported by several Investigators (Behera *et al*., 2005; Archana and Abraham, 2011; Ponmurugan *et al.,* 2016a).

Lichens are symbiotic organisms in which fungi in association with algae form an autotrophic single thallus. They are able to grow on rock (saxicolous), soil (terricolous), and bark (corticolous) as habitat across the world. A total population of about 22,000 lichen species are widespread in the world from which 2714 lichen species occur in India. It is estimated that around 557 lichen species are recognized as endemic to India. Lichens are known for unique secondary metabolites of about 1050 total compounds. Over 550 compounds are unique to only lichens. The derivatives of lichen compounds are fatty acids, macrolytic lactones, zeorins, pulvic acid derivatives, cumarone derivatives, dibenzofurans, depsides, depsidones, terpenoids, anthroquinone derivatives, steroids, carotenoids, and diphenyl ethers (Ayyappadasan *et al*., 2017).

Lichen compounds rendered several biological activities, and they are a promising anti-microbial agent alternative to antibiotics. Over 50% of the known lichen can have medicinal value (Vartia, 1973). Depending on the compounds enclosed in lichen, which encompass specific properties that are documented in the pharmacopoeias, the lichen compounds exert a wide variety of biological actions including anti-microbial, anti-viral, anti-inflammatory, anti-oxidant, analgesic, anti-pyretic, antiproliferative and anticancer activities, besides lichens also have anti-snake venom activity (Tanas *et al*., 2010; Shrestha *et al*., 2015). The secondary metabolites are

inevitable chemical substances, which assist lichen identification as well.

Parmotrema is a genus of lichen belonging to the family Parmeliaceae, kingdom Fungi, division Ascomycota, class Lecanoromycetes and order Lecanorales. The genus was circumscribed by Italian lichenologist Abramo Bartolommeo Massalongo in 1860. Members of the genus are commonly called ruffle lichens or scatter-rag lichens (Massalongo, 1860). *Parmotrema* is one of the largest genera of parmelioid core in the family Parmeliaceae in lichen group. *P. austrosinense, P. nilgherrense, P. hababianum, P. reticulatam* and *P. tinctorum* are the predominant species in Western Ghats of Tamil Nadu (Ponmurugan *et al*., 2016b). The thallus of *Parmotrema* spp. had 37.5% usnic acid and 23.5% lecanoric acid. The lichen extracts with protocetratic acid, physodalic acid, and physodic acid are organized in the medullary layer.

Materials and methods

Different foliose lichen species of *Parmotrema* were collected from Kodaikanal hills, Tamil Nadu, India for the present study. The height of the hills is about 2130 meters MSL. The species *P. reticulatam* and *P. nilgherrense* (*terriclous*) were scraped out of the soil substratum, while *P. tinctorum* (corticolous) and *P. hababianum* (corticolous) lichens were collected from the bark of the tree, and the species *P. austrosinense* (saxicolous) was collected along with the rock using a chisel.

The solvents such as petroleum ether (10.5%), ethyl acetate (15.5%) and chloroform (15.5%) were used as solvent extracts (Sawaya *et al*., 2004; Boyanova *et al*., 2005) for the present investigation. Phytochemical screening of the thallus of *Parmotrema* spp. revealed the presence of antimicrobial, antioxidant and anticancer compounds like usnic acid, lecanoric acid salazinic acid, atraonin, stictic acid, protolichesterinic acid, consalazinic acid, norstictic acid, lecanoric acid, lobaric acid and caperatic acid as per the report of Muller (2001).

Clinically challenging potential human bacterial species such as *Salmonella typhi, Escherichia coli, Bacillus subtilis, Klebsiella pneumonia, Pseudomonas fluorescence, Aeromonas hydrophylla* and *Staphylococcus aureus* were selected for the present study. The selected organisms are resistant to antibiotics commonly used for treatment of various diseases caused by these bacteria and the MAR index value also calculated. Mueller Hinton agar (Himedia) plates were prepared and seeded with 16 hour old cultures of the said pathogenic microorganisms. Before seeding the inoculum, the turbidity was adjusted to the turbidity of 0.5% of McFarland solution and challenged with different concentration of lichenic extracts by well diffusion method. The plates were incubated at 37°C under controlled condition and the zone of inhibition of pathogenic microorganisms due to solvent extracts containing lichenic phytochemical compounds was recorded subsequently.

Results and Discussion

Drug resistance of all known microorganisms to all existing anti-microbial agents is fully established. It is necessary to identify the new anti-microbial agents from medicinal plants and to determine which of the potential compound is more effective against unidentified pathogenic microorganisms. Therefore, this work will provide the baseline information for the future pharmacological studies. A bioprospection and antibacterial studies were carried out in five species of foliose lichen *Parmotrema* spp. The results showed that all the extracts of tested solvent extracts showed good activity against the tested pathogenic microorganisms.

Among *Parmotrema* spp. tested, *P. nilgherrense* showed greater activity followed by *P. austrosinense* than other *Parmotrema* spp. such as *P. hababianum, P. reticulatam* and *P. tinctorum* solvent extracts (Table 1-3). Similarly, among two solvent extracts tested for antibacterial activity, ethyl acetate extracts showed a higher growth inhibition zone around bacterial species followed by chloroform extracts. It was due to the impact of active ingredients like phenolic compounds and wide spectrum of secondary metabolites which were extracted with ethyl acetate rather than other solvents such as chloroform and petroleum ether. Since lichens are autotropic in nature due to synthesis of own food materials through algal components in the thallus and production of secondary metabolites by fungal partners. In addition, both lichen symbionts are physiologically and biochemically active in their life cycles (Poornima *et al*., 2018). The secondary metabolites produced by fungal partners give credence to its ethnopharmacological use as a remedy to treat infections and diseases caused by human pathogenic bacteria.

The results indicated that there was no significant difference among the bacterial species selected for the present study in terms of antibacterial activity. It is in accordance with the results of antibacterial activity of six types of tea leaf extracts due to mixed type vegetation and the study area locality (altitude and latitude) is near with one another (Drago *et al*., 2000; Ponmurugan *et al*., 2016a). According to Drago *et al*., (2000), it is very difficult in comparison the results of antimicrobial properties of medicinal plants using human pathogenic bacteria due to virulence nature of clinically isolated microorganisms and presence of secondary metabolites in medicinal plants including extraction of bioactive compounds using organic and inorganic solvents. Hegazi and El Hady (2001) reported that raw medicinal plant leaf materials tested against the clinically isolated microorganisms for inhibition activity might be minimal even though the high volume of extract was used.

Karagoz *et al*. (2009) investigated that lichens have showed a remarkable inhibition effect against selected bacterial species such as *Bacillus, Pseudomonas, E. coli, Streptococcus, Staphylococcus, Enterococcus, Mycobacterium, Bacillus licheniformis, B. megaterium, B. subtilis* and *S. aureus*.

An investigation was carried out by Ponmurugan *et al*. (2016a) to study the antibacterial activity of different green tea leaves such as mother, scale (cataphyll), first, second and third and buds against seven clinically isolated antibiotic resistant bacteria which revealed that a remarkable antibacterial activity of first and second green tea leaves followed by leaf bud was recorded. They were further mentioned that it was due to several biotic and abiotic factors including origin of tea leaves, vegetation habits, age of the leaves and extract preparation. Lichens could synthesize numerous secondary metabolites which comprise amino acid derivatives, sugar alcohols, aliphatic acids, macrocyclic lactones, mono-cyclic aromatic compounds, quinones, chromones, xanthhones, dibenzofuranes, depsides, depsidones, depsones, terpenoids, steroids, carotenoids and diphenyl ethers (Karagoz *et al*., 2009). The present investigation may be concluded that *Parmotrema* spp. was found to be effective in inhibiting the pathogenic microorganisms through antibacterial studies, which give a new dimension in green pharmacy in coming years.

Table 1: Antibacterial activity of *Parmotrema austrosinense* and *Parmotrema hababianum*[a]

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether 1%	Petroleum ether 2%	Ethyl acetate 1%	Ethyl acetate 2%	Chloroform 1%	Chloroform 2%
Parmotrema austrosinense								
Salmonella typhi	10.5	12.3	7.3	10.0	9.3	14.3	9.0	15.5
Escherichia coli	12.5	13.0	3.3	05.5	7.5	13.0	6.5	13.0
Bacillus subtilis	10.5	12.3	6.5	13.5	9.0	17.0	7.5	15.5
Klebsiella pneumonia	10.0	11.3	3.0	05.3	5.3	12.3	7.3	11.3
Pseudomonas fluorescence	14.5	13.5	3.3	06.5	11.3	17.7	10.0	14.3
Aeromonas hydrophylla	15.3	15.7	5.3	07.5	10.0	15.5	9.5	15.3
Staphylococcus aureus	15.3	14.5	5.3	07.7	11.0	13.5	9.5	15.3
Parmotrema hababianum								
Salmonella typhi	10.3	12.5	3.3	5.0	5.7	7.3	3.3	5.0
Escherichia coli	12.3	13.7	1.5	2.3	3.0	5.0	3.0	5.7
Bacillus subtilis	10.7	12.0	2.5	4.3	3.0	4.5	2.0	3.3
Klebsiella pneumonia	10.7	11.5	2.3	2.5	4.3	6.5	3.3	4.3
Pseudomonas fluorescence	14.5	13.3	2.3	3.5	4.3	6.3	2.5	4.5
Aeromonas hydrophylla	14.0	14.5	2.5	3.7	3.3	5.3	3.5	5.0
Staphylococcus aureus	14.0	14.0	2.5	3.5	4.5	7.3	3.0	5.3

[a] Values are the means of five replicates

Table 2: *Parmotrema reticulatum* and *Parmotrema tinctorum*[a]

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethyl acetate		Chloroform	
			1%	2%	1%	2%	1%	2%
Parmotrema reticulatum								
Salmonella typhi	12.5	12.3	6.6	9.3	9.7	14.3	7.5	13.5
Escherichia coli	12.3	13.5	5.5	8.3	6.5	11.3	6.5	12.5
Bacillus subtilis	11.3	12.7	5.5	9.5	9.5	15.5	6.5	11.3
Klebsiella pneumonia	11.0	12.5	4.5	7.5	7.5	13.0	4.0	07.3
Pseudomonas fluorescence	14.5	13.5	3.5	5.5	9.3	14.0	7.0	14.0
Aeromonas hydrophylla	15.7	15.7	4.5	7.5	9.3	13.0	7.3	13.5
Staphylococcus aureus	15.7	15.5	5.3	8.5	9.3	15.0	7.5	12.3
Parmotrema tinctorum								
Salmonella typhi	12.3	12.5	5.3	9.0	9.5	15.0	7.3	13.3
Escherichia coli	12.3	13.5	5.0	8.5	7.5	14.0	6.5	12.3
Bacillus subtilis	12.3	13.5	4.5	9.0	9.5	15.5	7.5	12.0
Klebsiella pneumonia	11.5	12.3	4.5	8.7	7.3	13.5	6.0	12.5
Pseudomonas fluorescence	14.5	13.5	5.3	9.5	10.0	15.0	7.0	14.3
Aeromonas hydrophylla	15.0	15.7	5.3	11.0	10.3	16.0	9.0	16.3
Staphylococcus aureus	15.0	13.7	6.3	11.0	10.5	16.0	9.0	16.0

[a] Values are the means of five replicates

Table 3: *Parmotrema nilgherrense*[a]

Bacteria	Zone of inhibition in mm							
	Streptomycin	Ciprofloxacin	Petroleum ether		Ethyl acetate		Chloroform	
			1%	2%	1%	2%	1%	2%
Parmotrema nilgherrense								
Salmonella typhi	10.5	12.7	6.3	9.7	9.7	14.3	9.5	15.7
Escherichia coli	12.3	13.3	6.3	9.5	6.5	11.5	6.5	12.0
Bacillus subtilis	12.3	15.5	5.3	9.0	9.7	15.0	6.0	11.5
Klebsiella pneumonia	12.7	12.0	4.0	7.5	7.5	15.0	4.5	07.7
Pseudomonas fluorescence	14.7	13.5	3.0	5.3	9.0	14.3	9.0	16.7
Aeromonas hydrophylla	15.3	15.3	4.5	8.7	9.7	13.3	7.3	13.0
Staphylococcus aureus	15.5	14.5	5.5	9.7	9.7	15.5	7.3	14.3

[a] Values are the means of five replicates

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